DNA double-strand breaks: their cellular and clinical impact?

Article (Unspecified)

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DNA, the central store of our genetic information, constantly incurs damage from agents generated within the cell as well as chemicals or radiation that arise externally. Of the many different classes of damage, a DNA double strand break (DSB) is arguably the most significant since, if unrepaired it can result in cell death and if misrepaired, can cause chromosomal translocations, an early step in the aetiology of carcinogenesis. Endogenously generated reactive oxygen species primarily induce base damage and single strand breaks and it is unlikely that DNA DSBs are directly induced to any significant extent. However, DSBs may arise indirectly from two closely located single strand breaks or during the repair of other lesions. They also arise when replication forks collapse, which may occur following attempted replication of single strand breaks or base damage. Indeed, a DSB is very likely the ultimate lesion induced by a wide range of DNA damaging agents. The enhanced levels of endogenous chromosome breakage or chromosome rearrangements that have been observed in cells that fail to repair DSBs efficiently attests to the fact that they represent a relatively frequently encountered endogenous lesion (Karanjawala et al., 1999). Despite the constant onslaught of endogenous oxidative damage as well as frequently encountered exogenous DNA damage, genomic changes are a rare event and cells can undergo multiple rounds of replication without wittnessing chromosomal alterations. This attests to the remarkable efficiency of the damage response pathways that function in response to DSB induction and their evolutionary importance.

Whilst somatic cells invest enormous resources into maintaining genomic stability, the development of the immune response is a contrasting process that necessitates the generation of a high level of genetic diversity. Curiously, the immune system exploits the same machinery that serves to prevent genomic instability in somatic cells to create diversity during development of the immune response (Bassing and Alt, 2004). Two important processes in this context are V(D)J recombination and class switch recombination, both of which involve programmed introduction of a DSB, that interestingly arise by highly distinct and defined mechanisms. NHEJ-dependent rejoining then occurs in a manner that creates new end junctions (Taccioli et al., 1993). These processes represent exquisite examples of adaptive evolution that is regulated in a highly tissue specific manner. The process of meiotic recombination represents another example where the purpose is to create a level of genetic reassortment, which again exploits the cellular machinery that aids the maintenance of genomic stability in somatic cells. Yet again, the cell introduces a DSB as a first step in the process.

The most significant of the external agents that induce DSBs is ionising radiation. Indeed, DSBs represent the most biologically significant lesion induced by ionising radiation. Understanding how cells respond to radiation exposure remains an important and timely issue. Despite the increased availability of chemotherapeutic drugs for the clinic, radiotherapy continues to represent the most widely used treatment for cancer, frequently being exploited as an adjunct to surgery. An ability to predict and assess a patient's response to radiotherapy, a holy grail for many decades, would enormously enhance the optimisation of radiotherapy regimes. Moreover, drugs targets that can

enhance the efficacy of radiotherapy are being actively sought. Additionally, the exploitation of radiation for medical benefits has increased significantly with the use of X-rays and for computer assisted tomography. Yet the harmful effects of exposure to low doses of radiation are still poorly understood. Finally, accidental or terrorist based exposure to radiation is a defined threat. Preparedness for such an event requires a knowledge of radiation responses, which encompasses the impact of DSB formation.

This issue of Oncogene Reviews focuses on the DNA damage response mechanisms that function to maintain genomic stability and cellular survival in response to DNA DSBs induction. The reviews encompass our current understanding of the basic mechanisms, the clinical impact when they fail to function efficiently and potential avenues by which our knowledge of the pathways might be exploited for clinical benefit. They range from biochemical studies dissecting the processes in vitro, to cellular studies using defective cell lines and siRNA strategies to down regulate components of the pathways, to the exploitation of mouse models, and last but of great significance, to the identification and characterisation of patients deficient in the pathways. This issue is timely since our knowledge of the basic damage response processes are is now reasonably well understood, providing an opportunity for exploitation within the clinic. Of all the developments in recent years, a highly important finding is that cells phosphorylate a variant of the histone H2A, H2AX, in the vicinity of a DSB that results in the accumulation of a range of damage response proteins (Paull et al., 2000). The break site is thus marked within the cell. This striking finding has facilitated the development of assays to detect DSB formation that are several orders of magnitude more sensitive than previous assays and, moreover, can be applied to a single cell. Furthermore, this observation is now being exploited in mice and indeed in humans to examine DSB formation in vivo(Lobrich et al., 2005). Another important consequence to emerge from our improved knowledge of the basic mechanisms is the identification of several human disorders deficient in DSB damage response processes (Ahnesorg et al., 2006; Buck et al., 2006; Moshous et al., 2001; O'Driscoll et al., 2001). Cell lines from such patients is are an important tool to aid further research but conversely, our increasing knowledge of the processes can aid the treatment of such patients.

The damage response to DSB formation encompasses pathways of DSB repair and signal transduction pathways that serve to establish cell cycle checkpoint arrest and/or activate apoptosis. Moreover, there is increasing evidence that damage response signalling communicates with the DSB repair machinery regulating at least some aspects of DSB repair (Deckbar et al., 2007). The two major DSB repair pathways are DNA non-homologous end-joining (NHEJ) and homologous recombination (HR). The phospho-inositol 3-kinase like kinase (PIKK), ataxia telangiectasia mutated (ATM), lies at the heart of the most significant signal transduction response to DSBs (Wyman and Kanaar, 2006). In mammalian cells, HR rarely uses a homologous chromosome as an undamaged template but instead exploits a sister chromatid that is present following replication. Consequently, HR functions solely in late S/G2 phase. In addition to the repair of replication fork associated DSBs, HR also effects, fork reversal to optimise repair of other lesions at the replication fork and to prevent replication fork collapse. Hence, NHEJ is the major pathway that repairs DSBs that are non-replication associated. Steve West and Dik Vvan Gent discuss our current understanding of the processes of HR and NHEJ, respectively, considering current topical aspects of the processes. ATM

dependent signalling is a complex process that involves sensor proteins, which recognise the damage and facilitate the activation of ATM, mediator proteins, which serve to amplify the ATM signal, transducer kinases, which relay the ATM signal to downstream proteins, which finally act as effectors of the endpoint, which can include cell cycle checkpoint arrest, apoptosis or DNA repair. An important issue in the field is the mechanism by which the DSB is sensed and how ATM is activated. Central to this issue is the role of the Mre11-Rad50-Nbs1 (MRN) complex. Several studies have suggested that MRN, rather than ATM itself, is the primary sensor of DSBs and is required to activate ATM (Uziel et al., 2003). However, it is difficult to distinguish activation of ATM signalling from amplification, a role played by the mediator proteins. Current evidence suggests that MRN has impacts on ATM signalling both upstream and downstream of ATM activation suggesting a role as a sensor but potentially also a role as a mediator protein. In this issue, Tanja Paull and Martin Lavin will discuss proteins that potentially regulate ATM activity, encompassing the role of the MRN complex. Tanja Paull has carried out excellent biochemical studies dissecting the process of ATM activation at the biochemical level whilst Martin Lavin has taken a cell based approach. A-T cells and patients, which harbour mutations in ATM, are exquisitely radiosensitive and indeed, A-T represents one of the most clinically radiosensitive conditions described. For some years, it was argued that cell cycle checkpoint defects where the primary cause of A-T radiosensitivity but more recently, ATM has also been shown to regulate a component of DSB repair (Riballo et al., 2004). Andre Nussenzweig in this issue will consider how the checkpoint and repair functions of ATM interplay during antigen receptor gene assembly, a process that functions during development of the immune response, to prevent the formation and proliferation of damaged lymphocytes., The dual deficiency of A-T cells a role that likely underlies the highly elevated frequency of lymphoid tumours in A-T patients. Now that we have a reasonable understanding of DSB repair, research is progressing to the next stage to understand how DSBs are repaired within the context of chromatin. This encompasses how chromatin can impede the DNA damage responses as well as understanding how the DNA damage responses effect chromatin modifications to deal with the problem. Indeed, current evidence suggests that a component of ATM signalling serves to modify chromatin structure to facilitate repair as well as to enhance the signal. Jessica Downs in this issue considers the DNA damage responses in the context of chromatin and the contribution of proteins that mediate changes in chromatin structure to the damage response.

The impact of DSBs on development human health and the consequence of defects in the damage response pathways are central, clinically important questions. These impacts extend not only from the role of the damage response pathways in maintaining genomic stability and hence in preventing carcinogenesis but additionally the pathways have roles roles that impact upon normal development. Jiri Bartek will consider how the damage response mechanisms act as a barrier to tumouroigenesis which encompasses roles in preventing of the formation of the initially damaged cell as well as in preventing the proliferation of pre-tumouroigenic cells. Jean-Pierre Villartay discusses the process of V(D)J recombination and the clinical impact of mutations in NHEJ proteins in causing immunodeficiency and developmental delay. Nijmegen breakage syndrome (NBS) is another disease associated with exquisite radiosensitivity and tumour predisposition. Martin Digweed discusses our current understanding of how a defect in NBS1, a factor

regulating ATM activity, manifests the particular sympton complex of NBS. Interestingly, one feature of patients deficient in NHEJ and NBS1 proteins is microcephaly, attesting to the importance of efficient DSB repair during neuronal development. This function of the damage response proteins is further considered by Peter McKinnon. If the DSB damage response proteins function as an important barrier to tumour progression, it is perhaps not suprising that some tumours will down or even up-regulate damage response proteins. Eckart Meese reviews genetic changes in glioblastoma, a tumour associated with pronounced radioresistance, and discusses evidence that such tumours can display alterations in proteins that impact upon NHEJ. Finally, Graeme Smith and Mark O'Conner consider the current status of approaches to exploit our knowledge of the damage response pathways as drug targets.

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